

Jurnal Teknologi, 48(F) Jun 2008: 51-60
© Universiti Teknologi Malaysia

MICROBIAL CONSORTIA FROM RESIDENTIAL WASTEWATER FOR BIOREMEDIATION OF PHENOL IN A CHEMOSTAT

NORHANI JUSOH^{1*} & FIRDAUSI RAZALI²

Abstract. The study aimed at evaluating the potential of employing microbial consortia from residential wastewater to degrade phenol, a common industrial pollutant. The consortia was subjected to high shock loads and different levels of phenol in continuous cultures. In the shock load study with 1000 ppm phenol in a 1.5 litre working volume chemostat, the culture with prior acclimatization to phenol demonstrated 17 times higher affinity towards phenol (i.e. $K_s = 173$ ppm). This resulted in double growth rate ($\mu_{max} = D_c = 0.04 \text{ h}^{-1}$) compared to non-acclimatized culture. However, the levels of phenol reduction in both cases were almost identical, approximately 60%. This indicated that the performance of different types of bacteria present in the non-acclimatized culture which was comparable to the acclimatized culture although with less number of microbe types. The effects of two loading concentrations, namely 200 ppm and 1000 ppm were examined at constant dilution rate of 0.02 h^{-1} . The feeding of 200 ppm exhibited 97% of phenol reduction in comparison to only 57% of 1000 ppm. This result reconfirmed the inhibitory effect of phenol reduction at concentration above 500 ppm as previously determined in batch culture. The study has successfully established key operating parameters and provided a useful guideline for future up-scaling studies and site trials.

Keywords: Acclimatization, continuous, degradation, phenol, residential wastewater

Abstrak. Kajian ini bertujuan menilai potensi penggunaan konsortium mikrob dari air sisa buangan kediaman untuk penguraian fenol, pencemar umum daripada industri. Konsortium tersebut diuji dengan muatan mengejut dan kepekatan fenol yang berbeza di dalam kultur selanjar. Dalam uji kaji muatan mengejut, 1000 bahagian per juta (bpj) fenol disuap ke dalam 1.5 liter isipadu kerja bioreaktor. Kultur yang teradaptasi menunjukkan afiniti terhadap fenol 17 kali ganda lebih tinggi ($K_s = 173$ bpj) dan mengandakan kadar pertumbuhan ($\mu_{maks} = D_c = 0.04 \text{ j}^{-1}$) berbanding kultur yang tidak teradaptasi. Pengurangan fenol untuk kedua-dua kes adalah hampir sama iaitu lebih kurang 60%. Penguraian oleh pelbagai jenis bakteria yang terdapat di dalam kultur yang tidak teradaptasi adalah setanding dengan yang teradaptasi walaupun dengan bilangan jenis bakteria yang lebih rendah. Kesan dua kepekatan masukan iaitu 200 bpj and 1000 bpj telah dikaji pada kadar pencairan malar 0.02 j^{-1} . Suapan pada kepekatan 200 bpj memberikan pengurangan sebanyak 97% berbanding hanya 57% untuk kepekatan 1000 bpj. Keputusan ini telah menyokong keputusan kesan rencatan pada kepekatan yang melebihi 500 bpj seperti yang diperolehi dalam kultur kelompok sebelum ini. Kajian ini telah berjaya mengenal pasti parameter yang penting untuk penguraian fenol dan amat berguna untuk skala yang lebih besar pada masa depan dan juga sebagai percubaan di tapak industri.

Kata kunci: Adaptasi, air sisa buangan kediaman, fenol, penguraian, selanjar

^{1&2} Department of Bioprocess Engineering, Faculty of Chemical & Natural Resources Engineering, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia

* Corresponding author: Tel: +60-7-5535805, Fax: +60-7-5581463, Email: noniey82@yahoo.com

1.0 INTRODUCTION

Phenol at high concentration is widely known as environmental pollutants due to commonly encountered in industrial effluents that include plastics manufacturing (600 – 2000 ppm), petrochemical manufacturing (50 – 600 ppm) and stocking production (6000 ppm) [1]. Efficient treatments of these wastewaters have been conducted by using several technologies including adsorption onto different matrices, solvent extraction, chemical oxidation and irradiation [2]. However, the existing removal methods have the inherent drawbacks due to the tendency of the formation of secondary toxic materials [3], complexity, and high cost of treatment [4]. Thus, in some cases, biological treatment has turned out to be a favourable alternative for phenol degradation.

Phenol degradation has been extensively investigated using variety of microorganisms including *Pseudomonas* sp. [3-7], *Rhodococcus* sp. [8], *Candida tropicalis* [9, 10] and *Acinetobacter* sp. [11]. Previous studies have shown that biodegradation of organic chemicals by using pure cultures usually can produce toxic intermediates. This problem may be overcome by the use of mixed cultures that have a wider spectrum of metabolic properties [5]. Therefore, in this study, mixed culture from residential wastewater was used as source of microorganisms to degrade phenol. Residential wastewater has been chosen because it contains diversify microorganisms as a result of wide variety of growth mediums including organic and inorganic matters as well as phenol substances. Phenol is released into residential wastewater as a product of metabolism in the urine and feces and phenol content in urine vary within the range of 5 to 55 mg/L [12].

In this work, phenol biodegradation was carried out in continuous culture and this mode closely mimics the real industrial processes. The objective of this work is to investigate the effect of acclimatization of cultures on continuous biodegradation with shock loading of phenol, and also to examine the tolerance of the microbial consortia towards inconsistent phenol, concentrations. The ability of acclimatized microbes to tolerate the shock load of phenol has been investigated since acclimatization stage was proved to overcome substrate inhibition problems that usually occurred in biodegradation at high concentration of phenol [13]. This is because certain enzymes in the microorganisms are induced during acclimatization stage, therefore, these microbes are available for next metabolism reaction [14].

2.0 MATERIALS AND METHODS

2.1 Experimental Setup

Batch and continuous biodegradations were performed in a bioreactor (B.BRAUN, Germany) with 1.5 litres working volume. The aeration rate was changed manually

to maintain the oxygen concentration at approximately 400 ppm. For continuous mode, an external multi channel pump (Cole Parmer, USA) was used to feed in the nutrient and to pump out the effluent from the bioreactor.

2.2 Culture and Media

Samples of wastewater were obtained from a local residential wastewater treatment plant and used as a source of the mixed culture. The culture was then enriched with LB medium that consists of 5 g/L yeast extract (CONDA, Spain), 10 g/L tryptone (CONDA, Spain), 15 g/L glucose (Sigma Chemical Co., USA) and 5 g/L sodium chloride (NaCl) (MERCK, Germany).

2.3 Acclimatization

Acclimatization of mixed cultures was carried out in bioreactor containing enriched microorganisms that was operated in batch mode with the addition of phenol at concentration level of 1000 ppm. The acclimatization stage has been carried out until complete removal of phenol.

2.4 Phenol Degradation

For the shock load study, 1000 ppm of phenol (Scharlau Chemie S.A, Spain) was incrementally supplied at 0.5, 1.0, 1.4, 1.8, and 2.0 ml/min into 1.5 L of acclimatized culture. The ability to withstand shock load of phenol was compared between acclimatized and non-acclimatized microorganisms. In a separate study, phenol has been supplied at different influent concentration namely 200 ppm and 1000 ppm at a constant flow rate of 0.5 ml/min. No nutrients was added, and air was supplied continuously during biodegradation process.

2.5 Analytical Methods

The microorganism growth was determined by measuring the optical density (OD) at 600 nm. For simplicity, one unit of optical density was assumed to correspond to approximately 1 g/L of biomass cell. Phenol concentration was determined by calorimetric method [15]. For each measurement, 2 ml of the sample was added to 0.3 ml of sodium carbonate (200 g/l) and 0.1 ml of Folin-Ciocalteau reagent. The absorbance was measured at 750 nm after 1 hour of incubation. A standard curve was prepared with concentration ranges from 0.002 to 0.01 g/L. A series of mass balance calculation were calculated for all continuous biodegradation to determine the average phenol concentration if biodegradation did not occurred. The average calculated phenol concentration was then compared with average phenol concentration in the purpose to obtain percent of phenol reduction.

3.0 RESULTS AND DISCUSSION

The competency of the uncharacterized microbial consortia was evaluated in continuous cultures supplemented with phenol as carbon source. Figure 1 shows the acclimatization stage that resulted in nearly complete reduction of phenol (93%) within 14 days of degradation. This result is comparable with result found by Gonzalez *et al.* [4] with complete reduction of phenol after 10 days of degradation with initial concentration of 1000 mg/L by using pure cultures of *Pseudomonas putida* ATCC 17484. This result proved that, robust mixed culture from residential wastewater have comparable performances as defined pure culture. This acclimatized consortia then was used in continuous biodegradation with shock loading of phenol as shown in Figure 2 and successfully reduced 61% of phenol concentration. The OD decreased as dilution rate is increased. Previous study also obtained that cell concentration decreased with incremental in dilution rate (D) during continuous biodegradation of phenol [6]. As a comparison, an experiment has been performed to study continuous biodegradation without acclimatization stage and the result is shown in Figure 3. These non-acclimatized consortia have resulted in 67% of phenol reduction. Incremental loading rate has apparently decreased the stability of the continuous culture for both experiments. Microbes managed to degrade phenol at low dilution rates, namely 0.02 h^{-1} and 0.04 h^{-1} . Further increase of dilution rate at 0.056 h^{-1} has resulted in a washout, and consequently increased the phenol level up to 200 ppm. Hensel and Straube [8] also observed that the stability of the system decreased with increasing dilution rate.

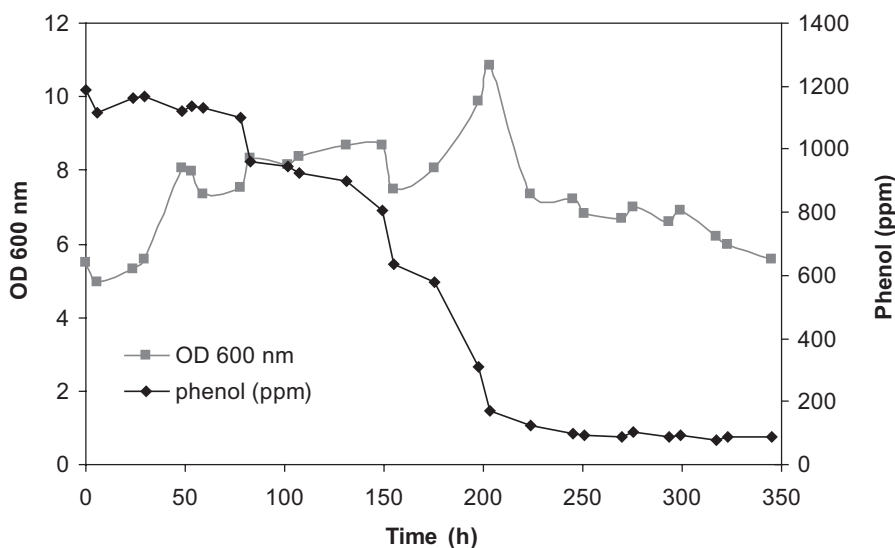


Figure 1 Phenol degradation during acclimatization stage

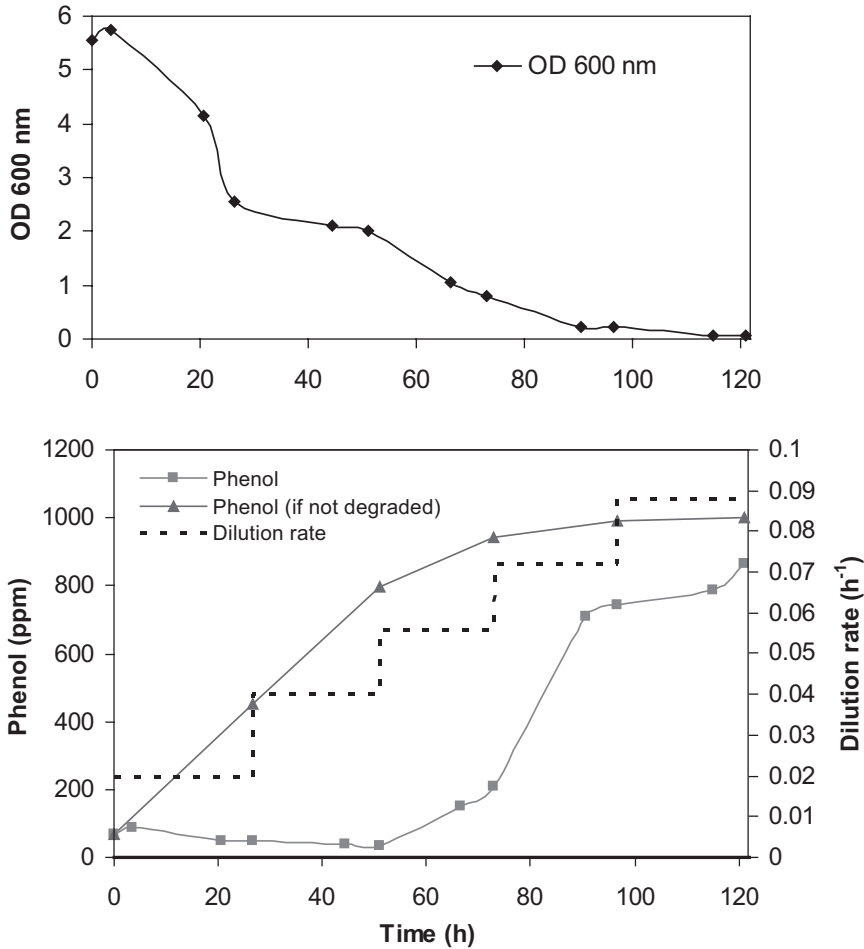


Figure 2 Continuous biodegradation of acclimatized microorganisms

We discovered that the critical dilution rate, D_c , of acclimatized culture was 0.04 h^{-1} , which was twice of non-acclimatized one, which was less than 0.02 h^{-1} . Table 1 summarized the kinetic data including substrate utilization constant (K_s), maximum specific growth rate (μ_{max}) and yield factor ($Y_{x/s}$) of these experiments. The acclimatized culture also indicated 17 times lower saturation constant, K_s (i.e. 173 ppm) than the non-acclimatized one. It is evident that the acclimatization step has significantly improved the microbe's affinity towards phenol. Despite this, no significant difference of average percentage of phenol reduction was witnessed between both cultures at the end of the process. The most possible explanation is that the performance of different types of bacteria present in the non-acclimatized culture was comparable to the acclimatized one, but with less number of microbe types. This was confirmed by

an unpublished result in a separate study in our laboratory indicating the reduction from five types to only two of residential wastewater microbes after treatment with phenol. Although both non-acclimatization and acclimatization step have resulted in the same average of phenol reduction, improvements in survival rate and affinity level offer more advantages especially at lengthy continuous degradation process.

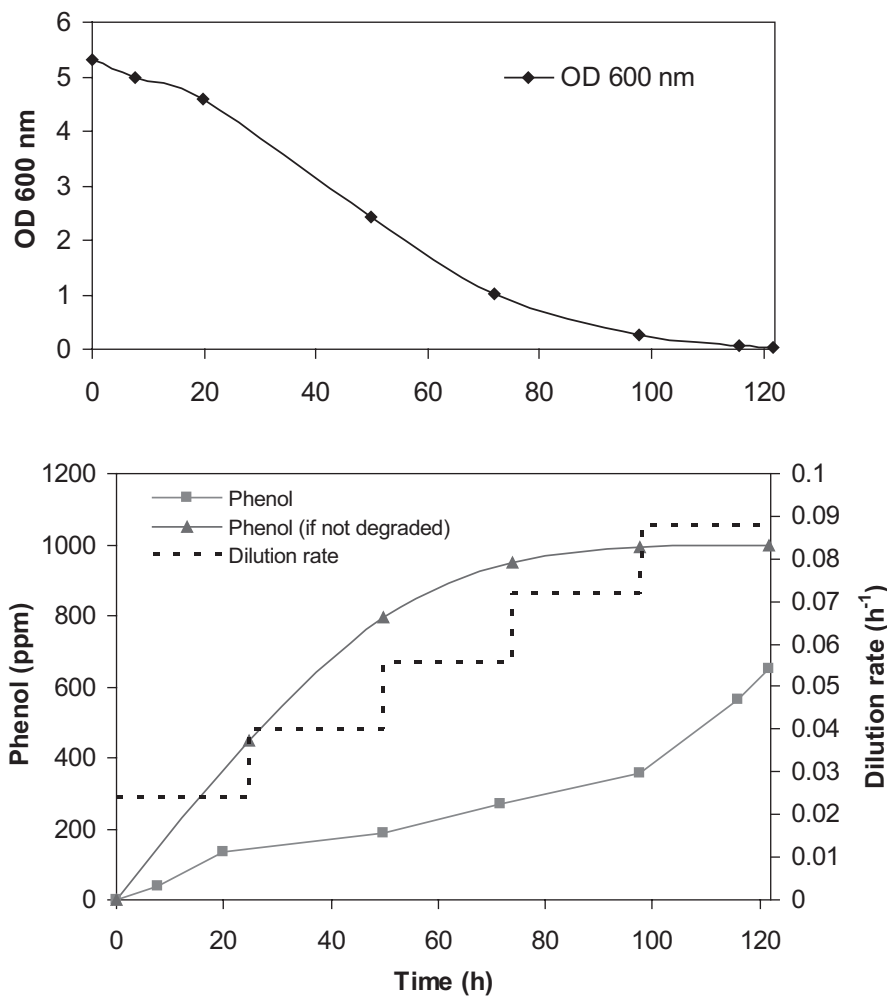


Figure 3 Continuous biodegradation of non-acclimatized microorganisms

Inconsistent loading strength is a common phenomenon in the actual waste treatment sites. Investigation on varying loading concentration on the degradation kinetic may determine its maximum tolerance level. It is a well-known fact that most degradation obeys the first order kinetics, where the rate of reaction is a function to its initial concentration. The influence of phenol initial concentration

on the degradation kinetic was investigated firstly in batch, and then extended to continuous culture. Results in batch cultures at three different initial phenol concentrations, namely 200, 500, and 1000 ppm indicated that both degradation rate (i.e. 2.06 ppm/h) and specific growth rate (i.e. 0.035 h⁻¹) at 500 ppm phenol were at the highest, as shown in Table 2. Our data is apparently in agreement with previous study by Bandyopadhyay *et al.* [3] that inhibitory effects of phenol as substrate have become predominant above concentration of 500 ppm of phenol by using *Pseudomonas putida* (MTCC 1194).

Table 1 Kinetics parameters of continuous biodegradation

Study	Microbes	μ_{max} (h ⁻¹)	K_s (ppm)	$Y_{x/s}$ (gX/gS)
Feitkenhauer <i>et al.</i> [16]	<i>Bacillus thermoleovorans</i>	0.9	NA	0.84
Lallai <i>et al.</i> [17]	Aerobic mixed culture	0.34	1.61	0.47
Seker <i>et al.</i> [18]	<i>Pseudomonas putida</i>	0.569	18.539	0.521
Present study	Non-acclimatized mixed culture	0.68	3024	8.32 × 10 ⁻⁵
Present study	Acclimatized mixed culture	0.0822	173	0.479

Table 2 Effect of initial concentration on phenol degradation

Initial concentration (ppm)	200	500	1000
Degradation rate (ppm/h)	0.59	2.06	1.8
Growth rate, μ (h ⁻¹)	0.005	0.035	0.006
Reduction (%)	41	91	60

Figures 4 and 5 show a time-course profile of continuous phenol degradation with feeding concentration of 200 ppm and 1000 ppm, respectively. In continuous cultures at dilution rate of 0.02 per hour, the feeding of 200 ppm phenol gave 97% of phenol reduction in comparison to only 57% at 1000 ppm. This result shows the dependence of the effluent concentration on feed concentration. This observation matched well with a published data reported by Beltrame *et al.* [19] found that substrate concentration in the effluent increases proportionally to feed

concentration. This kinetic anomaly is due to substrate inhibition by some secondary reaction products. However, the total phenol removal rate (defined as difference of phenol concentration between influent and effluent, multiplied by dilution rate) [20] at 1000 ppm was three time higher than at 200 ppm as shown in Table 3. Should higher loading concentration, i.e. 1000 ppm is unavoidable, lower dilution rates may compensate the harsh effect of phenol.

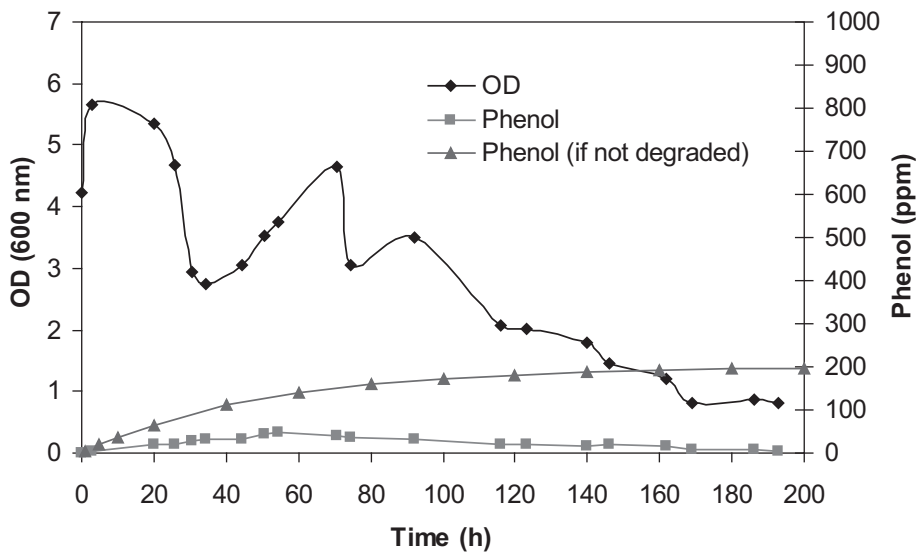


Figure 4 Continuous biodegradation with 200 ppm of influent concentration

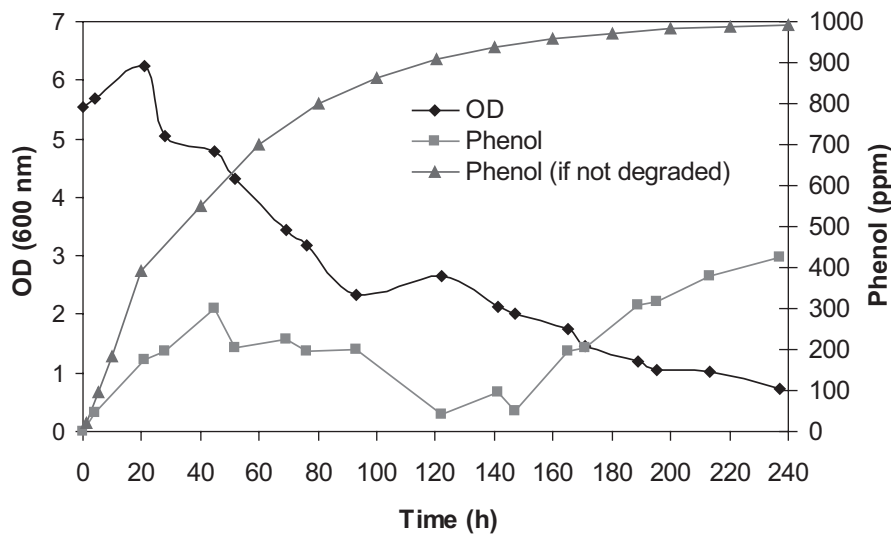


Figure 5 Continuous biodegradation with 1000 ppm of influent concentration

Table 3 Effect of influent concentration on continuous biodegradation

Influent concentration (ppm)	D (h ⁻¹)	Effluent concentration (ppm)	Phenol reduction (%)	Removal rate (ppm/h)	References
200	0.02	6.84	97	3.863	Present study
1000	0.02	426	57	11.48	Present study
1000	0.144	170	83	119.52	Lakhwala <i>et al.</i> [21]
1000	0.072	15	98.5	70.92	Livingstone [22]

4.0 CONCLUSION

Acclimatization of microorganisms to phenol has improved microbe's survival rate as well as affinity to the substrate. No significant improvement in phenol reduction shown by acclimatized culture compared to non-acclimatized one. Phenol concentrations above 500 ppm might inhibit the culture and resulted in lower rate of phenol reduction. This is true for both batch and continuous modes. In short, in this study we have successfully established key operating parameters (i.e. dilution rates, maximum specific growth rate and feeding concentration) for phenol degradation using microbes from wastewater and provide useful guideline for future scaling works.

ACKNOWLEDGEMENTS

We would like to thank all technicians and postgraduates in the Department of Bioprocess Engineering for their contributions to this research. We also like to express our gratitude to the UTM's Research Management Centre for financing this study through a short term grant (Vot. 75200) as well as providing the scholarship.

REFERENCES

- [1] Sitting, M. 1975. *Environmental Sources and Emissions Handbook*. N.J., USA: Noyes Data Corp.
- [2] Spiker, K., L. Crawford, and C. Thiel. 1992. Oxidation of Phenolic and Non Phenolic Substrates by the Lignin Peroxidase of *Streptomyces viridosporous* T7A. *Applied Microbial Biotechnology*. 37: 518-523.
- [3] Bandhyopadhyay, K., D. Das, P. Bhattacharyya, and B. R. Maiti. 2001. Reaction Engineering Studies on Biodegradation of Phenol by *Pseudomonas putida* MTCC 1194 Immobilized on Calcium Alginate. *Biochemical Engineering Journal*. 8: 179-186.

- [4] Gonzalez, G., M. G. Herrera, M. T. Garcia, and M. M. Pena. 2000. Biodegradation of Phenol in Continuous Process: Comparative Study of Stirred Tank and Fluidized Bed Reactors. *Bioresource Technology*. 76: 245-251.
- [5] Monteiro, A. A. M. G., R. A. R. Boaventura, and A. E. Rodrigues. 2000. Phenol Biodegradation by *Pseudomonas putida* DSM 548 in a Batch Reactor. *Biochemical Engineering Journal*. 6: 45-49.
- [6] Kim, J. H., K. K. Oh, S. T. Lee, S. W. Kim, and S. I. Hong. 2002. Biodegradation of Phenol and Chlorophenols with Defined Mixed Culture in Shake Flasks and a Packed Bed Reactor. *Process Biochemistry*. 37: 1367-1373.
- [7] Sokol, W. and C. L. C. Migiro. 1995. Controlling a Continuous Stirred Tank Bioreactor Degrading Phenol in Stability Range. *The Biochemical Engineering Journal*. 62: 67-72.
- [8] Hensel, J. and G. Straube. 1990. Kinetic Studies of Phenol Degradation by *Rhodococcus* sp. P1 II. Continuous Cultivation. *Antonie Van Leeuwenhoek*. 57: 33-36.
- [9] Chen, K. C., Y. H. Lin, W. H. Chen, and Y. C. Liu. 2002. Degradation of Phenol by PAA-immobilized *Candida tropicalis*. *Enzyme and Microbial Technology*. 31: 490-497.
- [10] Tsai, S. C., L. D. Tsai, and Y. K. Li. 2005. An Isolated *Candida albicans* TL3 Capable of Degrading Phenol at Large Concentration. *Biosc. Biotechnol. Biochem*. 69: 2358-2367.
- [11] Hao, O. J., M. H. Kim, E. A. Seager, and H. Kim. 2002. Kinetics of Phenol and Chlorophenol Utilization by *Acinetobacter* species. *Chemosphere*. 46: 797-807.
- [12] Sittig, M. 1980. Phenol in Priority Toxic Pollutant - Health Impacts and Allowable Limits. *Environment Health Series*. 1: 300-304.
- [13] Lob, K. C. and P. P. Tar. 2000. Effect of Additional Carbon Source on Biodegradation of Phenol. *Bulletin of Environmental Contamination and Toxicology*. 64: 756-767.
- [14] Kumar, A., S. Kumar, and S. Kumar. 2005. Biodegradation Kinetics of Phenol and Catechol Using *Pseudomonas putida* MTCC 1194. *Biochemical Engineering Journal*. 22: 151-159.
- [15] Box, J. D. 1981. Investigation of the Folin – Ciocalteu Phenol Reagent for the Determination of Polyphenolic Substances in Natural Waters. *Water Research*. 17: 511-524.
- [16] Feitkenhauer, H., S. Scnicke, R. Muller, and H. Markl. 2003. Kinetics Parameters of Continuous Cultures of *Bacillus thermoleovorans* sp. Degrading Phenol at 65°C. *Journal of Biotechnology*. 103: 129-135.
- [17] Lallai, A. and G. Mura. 1994. Steady-state Behaviour of an Aerobic Mixed Culture Growing on Phenol in a Continuous Stirred Reactor. *The Chemical Engineering Journal*. 53: B47-B55.
- [18] Seker, H., H. Beyenal, B. Salih, and A. Tanyaloc. 1997. Multi Substrate Growth Kinetics of *Pseudomonas putida* for Phenol Removal. *Applied Microbial Biotechnology*. 47: 610-614.
- [19] Beltrame, P., P. L. Beltrame, and P. Carniti. 1984. Influence of Feed Concentration on the Kinetics of Biodegradation of Phenol in a Continuous Stirred Reactor. *Water Research*. 18: 103-407.
- [20] Mordocco, A., C. Kuek, and R. Jenkins. 1999. Continuous Degradation of Phenol at Low Concentration Using Immobilized *Pseudomonas putida*. *Enzyme Microb Biotechnol*. 25: 529-536.
- [21] Lakhwala, F. S., B. S. Goldberg, and S. S. Sofer. 1992. A Comparative Study of Gel Entrapped and Membrane Attached Microbial Reactors for Biodegrading Phenol. *Bioprocess Engineering*. 8: 915-926.
- [22] Livingstone, A. G. 1993. A Novel Membrane Bioreactor for Detoxifying Industrial Wastewater. I. Biodegradation of Phenol in a Synthetically Concoted Wastewater. *Biotechnology Bioengineering*. 41: 915-926.